AD-A283 076

First Year Progress Report

DTIC ELECTE AUG 1 0 1994 F

Submitted to:

The Office of Naval Research

Project Title:

Estuarine Colloids: Sorption Capacity, Colloid Facilitated Transport and Bioavailibility

Submitted by:

Peter Santschi and Gary A. Gill
Department of Marine Science
Texas A & M University
Galveston, TX 77551

Mark Benfield
Woods Hole Oceanographic Institution
Woods Hole, MA

This document has been approved for public release and sale; its distribution is unlimited.

July 21, 1994

94 8 08 081

94-25008

408934

DTIC QUALITY INSPECTED 1

INTRODUCTION

The central objective of this research is to investigate the role of estuarine colloids as a transport medium in estuaries and to organisms, and their possible protective role to bioaccumulation processes and bioavailibility. Our primary hypothesis is that particle-reactive, strongly hydrolyzed metal ions (A-type) are primarily associated with hydrophilic colloids, which decreases their bioavailibility to organisms. A corollary hypothesis is that transition and B-type metal ions, organo-metal, and organo-metalloids are primarily associated with lipophylic colloids, which can increase their bioavailibility to organisms.

The primary objectives of this work are:

1. Elucidate the environmental fate of colloidally bound trace metals and metalloids in four contrasting Texas estuaries (Trinity River /Galveston Bay, Laguna Madre, Corpus Christi and Sabine Lake Estuaries).

2. Determine the toxicity/bioavailibility of colloidally-bound trace elements and metalloids to a marine shrimp, *Penaeus setiferus*.

3. Characterization of estuarine colloidal matter. General acid-base properties and the partitioning of trace metals and metalloids to estuarine colloidal matter is to be determined using radiotracer methodology.

Described briefly below is the progress we have made on each of these objectives.

I. THE ESTUARINE TRANSPORT OF COLLOIDALLY BOUND TRACE ELEMENTS

Initial efforts in this area investigated the suitability of obtaining reliable measurements of colloidal trace elements. Colloid isolation techniques have not been critically evaluated for collection and contamination artifacts with respect to trace element measurements. We conducted experiments on sample water, ultra-filtered water and retentate water to investigate:

- Sample processing blank levels for several contamination prone trace elements (Hg, Pb, and Zn)
- Artifacts associated with sample holding time prior to ultra-filtration
- Trace element and DOC "breakthrough" during the filtration process

Several field sampling trips to Texas estuaries were completed within the last year to investigate the role of colloids in the estuarine transport of trace metals and metalloids. A summary of the field collections that were accomplished is given in Table 1.

Table 1.
Field Collections

Sampling Site	Date	Samples Collected
Galveston Bay	July 1993	7
Corpus Christi	Oct. 1993	4
Sabine Bay	Nov. 1993	5
Corpus Christi	Apr. 1994	5
Galveston Bay	May 1994	7

At each sampling site collections were made for analysis of total metals or metalloids (unfiltered), dissolved (0.45 μm pore size), colloidal samples, total dissolved particulates, salinity, nutrients, DOC, and chlorophyll. The trace elements to be analyzed include Hg, As, Se, Al, Mn, Fe, Ag, Cd, Cu, Ni, Pb, and Zn. The procedures used are described below.

Sample Collection

Water samples were collected following techniques described by Flegal et al (1991) Briefly, samples are obtained using a peristaltic pump (Masterflex, Cole Palmer) fitted with acid-cleaned C-Flex tubing in the pump head. Water was drawn from a depth of about 30 cm through acid-cleaned Teflon tubing. The tubing is held in place by attaching it to a fiberglass pole which is oriented 3 m upstream of the boat's drift. Water is filtered as it is collected by pumping it through an acid-cleaned 0.4 μm pore size, polypropylene/polyethylene, in-line cartridge filter (MSI) directly into acid-cleaned 500 mL low density polyethylene (LDPE) bottles, 1 L Teflon bottles, or 20 L Teflon bags supported in high density polyethylene bottles (NOWPACK, Bag-in-a-Bottle, Berghoff). Sample bottles are placed in double plastic bags, placed in ice, and kept dark until analysis. Parallel filtration was carried out to collect suspended solids by drawing water through 47 mm diameter, 0.45 μm pore size, polycarbonate membranes (Nuclepore) loaded in TEFZEL filter holders (Savillex). The sampling units were prepared and samples were processed in a Class-100 trace metal clean laboratory, using high purity reagents and triple distilled, sub-boiling quartz distilled acids and solvents.

Sample Treatments

Water samples for total and dissolved measurements (except colloidal samples) were preserved immediately after collection by acidification with 2 ml ultrapure Q-HNO_3 (or Q-HCl) (triple distilled) per liter of sample within a Class-100 clean lab. Filters were unloaded from their Teflon assemblies and transferred to acid cleaned 30 ml Teflon Oak Ridge tube and followed by a three step digestion protocol described by Landing and Lewis (1991). The first digestion consisted of 25% acetic acid at room temperature for 4 h. This digestion is used to release adsorbed cations, carbonates, and reactive Mn oxyhydroxides. It also release Fe carried as amorphous oxides. The

oxides. The second digestion consisted of a 2M HCl/1M HNO_3 mixture at room temperature for 4 h. This digestion will release strongly bound cations not released by the acetic acid treatment and will attack certain types of aluminosilicates and crystalline Fe oxides. The third digestion was carried out at 60°C in a ultrasonic bath for 60 min. then in a Microwave digestion system (CEM model MDS-81D) using a mixture of HCl, HNO_3, and HF. This digestion will dissolve aluminosilicates and many accessory minerals not released by the second digesting acid.

Filtered water samples were digested in their original bottles (1 L Teflon and 500 mL LDPE) using the preservation acid and ultra-sonification for 60 min. at 60°C and UV-irradiated for 12 hr's under a bank of 25 W ultraviolet lamps. The trace metals were preconcentrated using Chelex-100 extraction method which described by Bruland et al. (1985) and Pai et al. (1988). Elemental concentrations were measured in triplicate in acid-washed Teflon autosampler cups using a Perkin-Elmer Zeeman 5100 PC atomic absorption spectrophotometer equipped with pyrolyzed furnace tubes and L'Vov platforms. Column yields were monitored by parallel measurement of certified reference sea waters (CASS-2 and SLEW-1, National Research Council of Canada).

20 L filtered water samples stored in Teflon bags were ultra-filtered in a Class-100 laminar flow clean bench through an Amicon cross-flow spiral cartridge (Model S10N1, nominal molecular weight cut-off 1,000 Daltons) as soon as possible. About 500 mL colloidal fraction samples were collected in a acid-cleaned Teflon bottles. Aliquots of the final colloidal solution (~ 100 mL) were acidified with 5 mL of triply quartz distilled nitric acid (Q-HNO_3) and processed by the same procedure as described above for elemental analysis. 500 mL to 1 L ultra-filtered water was also collected and analyzed. Prior to ultra-filtration the membrane cartridge was cleaned as follows: 5% Micro solution recycled for 2 hours, followed by processing 40 L of deionized water, DW, (18 Mohm water from a Nanopure system, Barnstead), 0.1 N Q-HCl recycled for 1 hour, follow by processing 20 L of DW, 1% Q-NH_4OH recycled for 1 hour, followed by processing 20 L of DW, 1% Q-HAc recycled for 1 hour, followed by 40 L of DW. During the last water flushing the water from both recycle and permeate were monitored for Zn by GF-AAS until concentrations fell below the detection limit (< 50 ng/L) by direct injection. At this point 500 mL aliquots were collected for blank determinations.

Results

A good illustration of the results obtained to date is given the data collected in Galveston Bay. The sampling stations in Galveston Bay that we have chosen are depicted in Figure 1. As we hypothesized, significant amounts of transition (e.g. Cu (II) and Ni) and type-B (e.g. Hg (II) and Pb (II)) trace metals are colloidally-bound in estuaries (Figures 2 and 3). For these trace elements, the percentage of colloidally-bound trace element is highest at the freshwater end-member, but remains a significant portion of the dissolved fraction through out the estuary. This preliminary information suggests that colloids serve an important role in the transport of type-B and transition metal trace elements in estuaries. We are currently analyzing these samples for the metalloids As and Se and the type-A trace element Al (III) to determine whether they have a significant colloid fraction in estuaries. We anticipate that this latter analytical work will be completed for presentation at the MEQ meeting this fall.

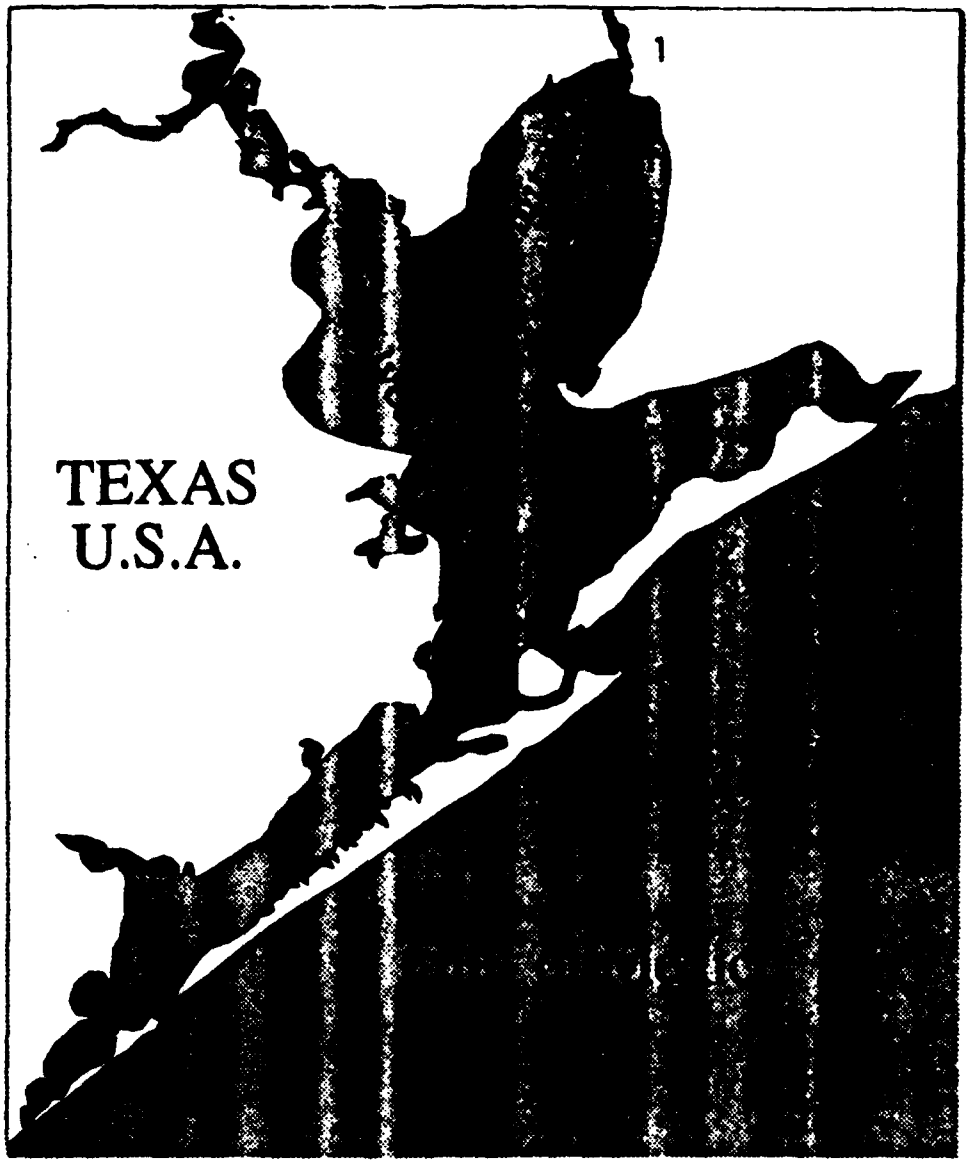

Figure 1. Location of Sampling Stations in Galveston Bay

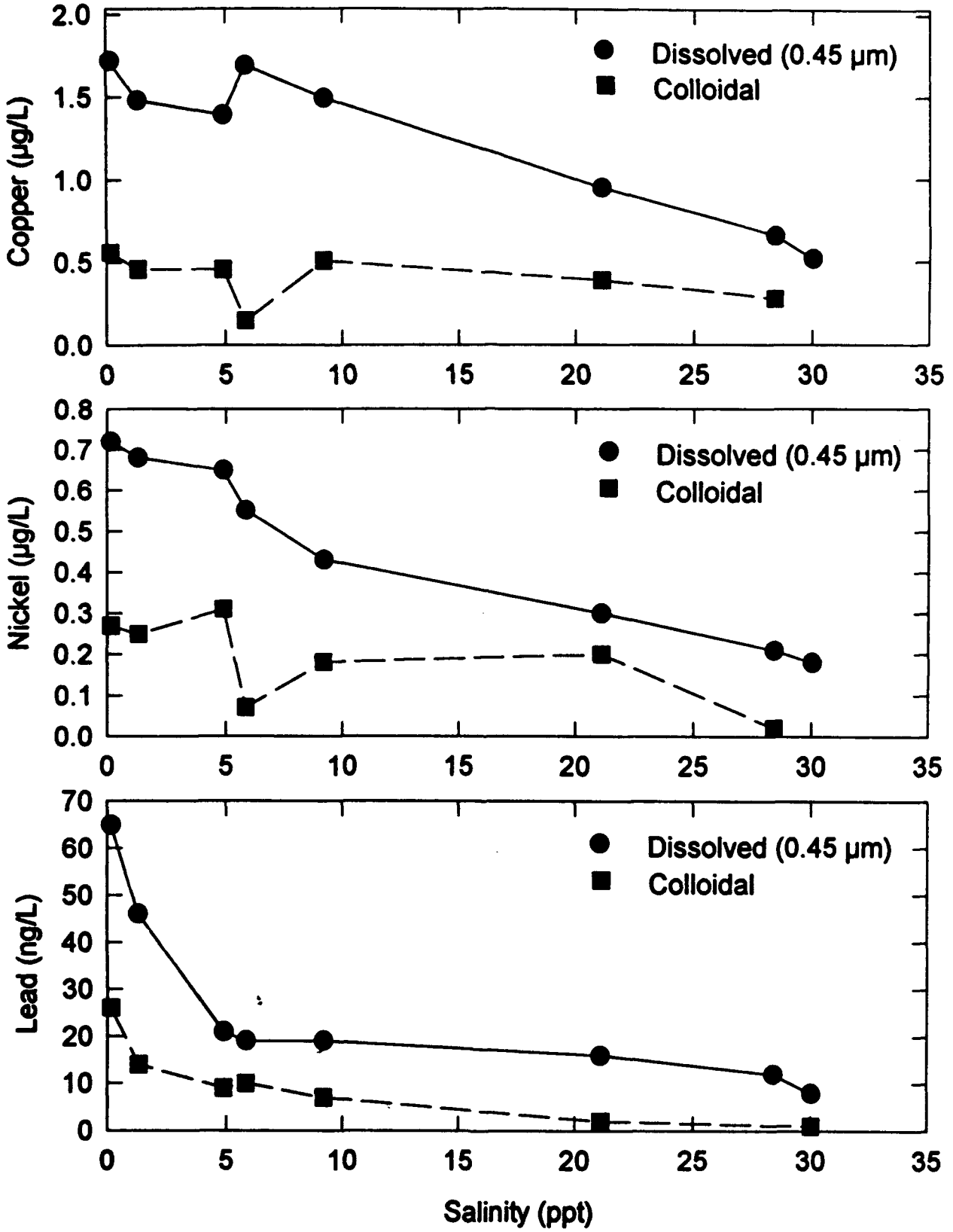

Figure 2. Dissolved and colloidal copper, nickel, and lead in Galveston Bay, July 1993

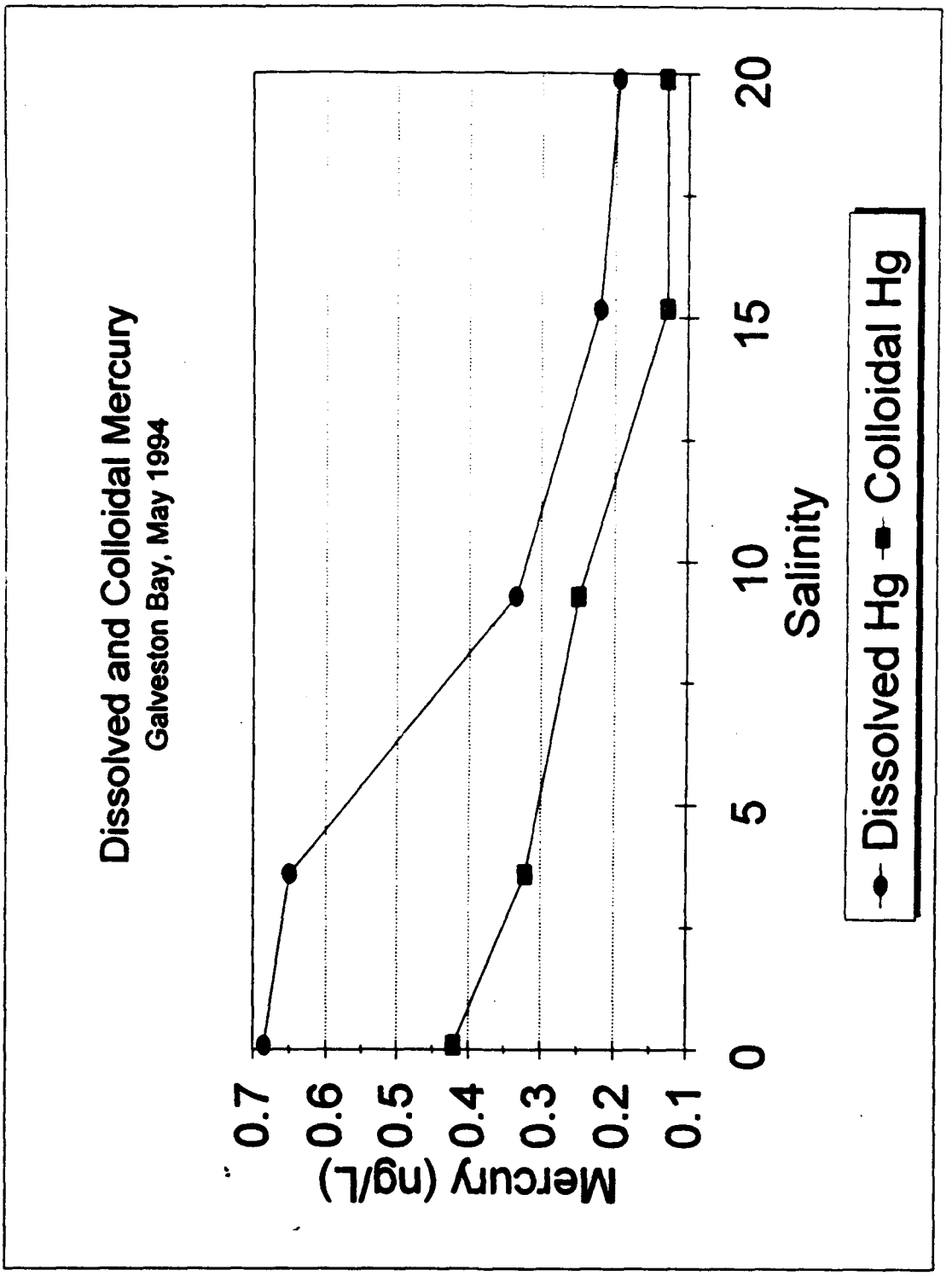

Figure 3. Dissolved and colloidal mercury in Galveston Bay.

II. TOXICITY/BIOAVAILIBILITY OF COLLOIDALLY-BOUND TRACE ELEMENTS

The objectives of this component of the project focus on the role of colloids in mediating the uptake of adsorbed heavy metals and metalloids and we are specifically interested in:

- How does sequestration of trace metals and metalloids into colloids modify bioaccumulation and subsequent depuration rates?
- Where in the shrimp (hepatopancreas, abdominal muscle, carapace) do colloidally-bound and free-ion forms of metals and metalloids accumulate?
- Do colloids provide any nutritive value to the early life-history stages (protozoeal, mysis, postlarvae) of shrimp?

Our animal model is the juvenile stage of the commercially and ecologically important white shrimp Penaeus setiferus which is available in Galveston Bay from late-May through September.

Our 1992/94 experiments began in the fall of 1993 with experimental design, acquisition of materials and construction of clean bench and animal holding systems. Initial radiotracer studies employing 60-Co were initiated in January 1994 with a comparably sized test species (grass shrimp Palaemonetes pugio) because the desired penaeids had not yet entered the bay. Subsequent experiments with Penaeus setiferus were initiated in early June and are ongoing at the time of this report.

Bioavailibility Experiments

The initial bioavailibility studies with grass shrimp were conducted in January 1994 in-order to develop the protocols for subsequent tests with white shrimp and evaluate the uptake rates of 60-Co in free-ion and colloid-ion complexes. Treatments (free ionic 60-Co, 60-Co complexed with colloids) were prepared in replicate (n=3) acid-washed 500 mL Teflon chambers containing 300 mL of test solution. Colloids were concentrated from Galveston Bay water (200 mg C/L) and sufficient concentrate was added to the colloid-ion chambers to achieve a concentration of 20 mg C/L. Each chamber was then spiked with 4.5×10^{-2} μCi of 60-Co. Six grass shrimp (mean mass = 0.1 g) were randomly assigned to each chamber and whole body counts of activity were conducted on one randomly selected animal from each of the three replicated chambers at intervals of 0, 6, 12, 24, 48 and 72 h (Fig. 4). Water samples (1 mL) were also counted at these times. Water samples were collected from a third set of replicate chambers containing colloid-ion complex without shrimp to assess loss of ion to the chamber walls.

Uptake rates appeared to be similar in both the free-ion and colloid-ion treatments, however, greater quantities of radiolabel were incorporated into the shrimp when ions were complexed with colloids (Fig.4). The early mortality of shrimp in the free-ion treatment was a consequence of slightly depressed pH levels in that treatment which resulted from the addition of the acidified spike. Buffering by colloids probably prevented such conditions in the colloid treatment. 60-Co uptake appeared to stabilize by 24–48 h and subsequent uptake studies have, and will be

conducted for 48 h. This experiment allowed us to refine the protocols which we currently use with white shrimp.

Experiments with juvenile white shrimp began on June 15, 1994. As of June 30, 1994, we have conducted uptake experiments with 60-Co, 110-Ag and 51-Cr isotopes and a preliminary nutritive study with postlarval P. setiferus to evaluate the influence of colloids on growth and survival.

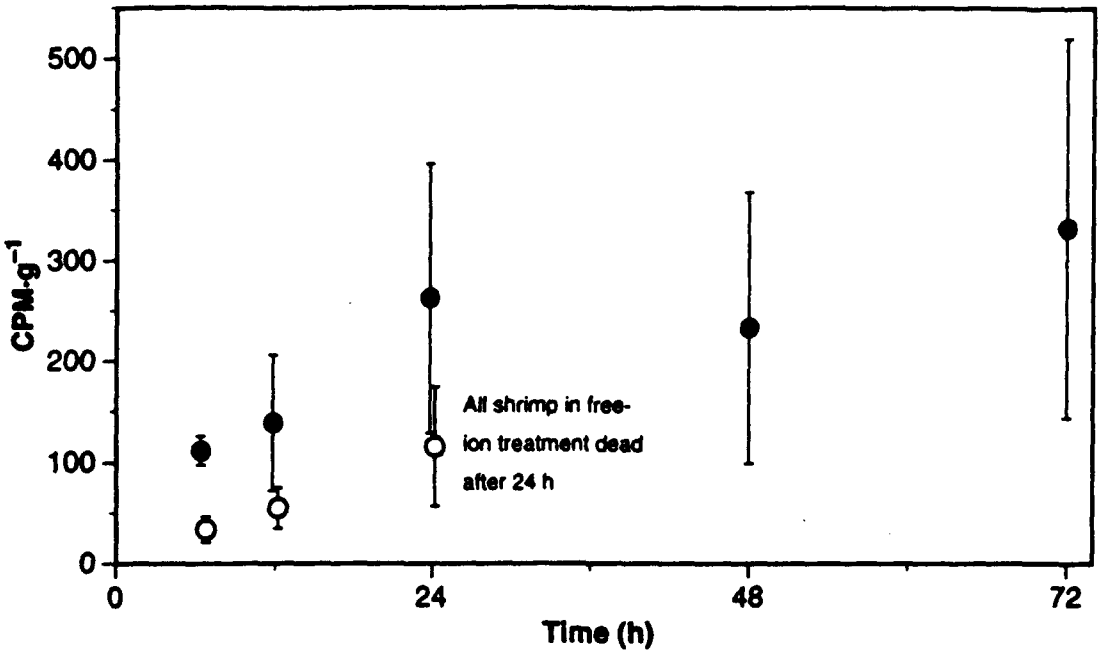

Figure 4. Mean (n=3) accumulation of 60-Co by grass shrimp Palaemonetes pugio in free-ion (o) and colloid-ion complex treatments (●) over 72 hours. 6–24 h samples have been artificially offset to avoid overlap of error bars. Error bars reflect ± 1 standard deviation.

Our protocol has been refined by allowing the ion and colloids to complex and then dialyze across a 1000 MW membrane for 24 h before addition to the experimental chambers. This step was added in-order to minimize the free-ion residue in the colloid-ion complex treatments. Sea water (20‰) controls have been added to evaluate natural mortality rates and background radioactivity levels. Our general protocol is illustrated in Fig. 5.

Although the majority of our samples are awaiting counting, several modifications to our protocols have become necessary. We are attempting to conduct all treatments at environmentally realistic (low ppt range) concentrations of metals. Consequently, relatively low levels of activity have been added to each treatment to meet these concentration criteria and this has resulted in low sample activities which require excessively long counting times. It appears essential that we employ isotopes with high specific activities and carrier-free isotopes when available. Both the 60-Co and 110-Ag studies will be repeated using spikes with higher specific activities. Our current studies of 51-Cr employ a high activity spike (2.54 µg Cr·mCi/L) which will reduce

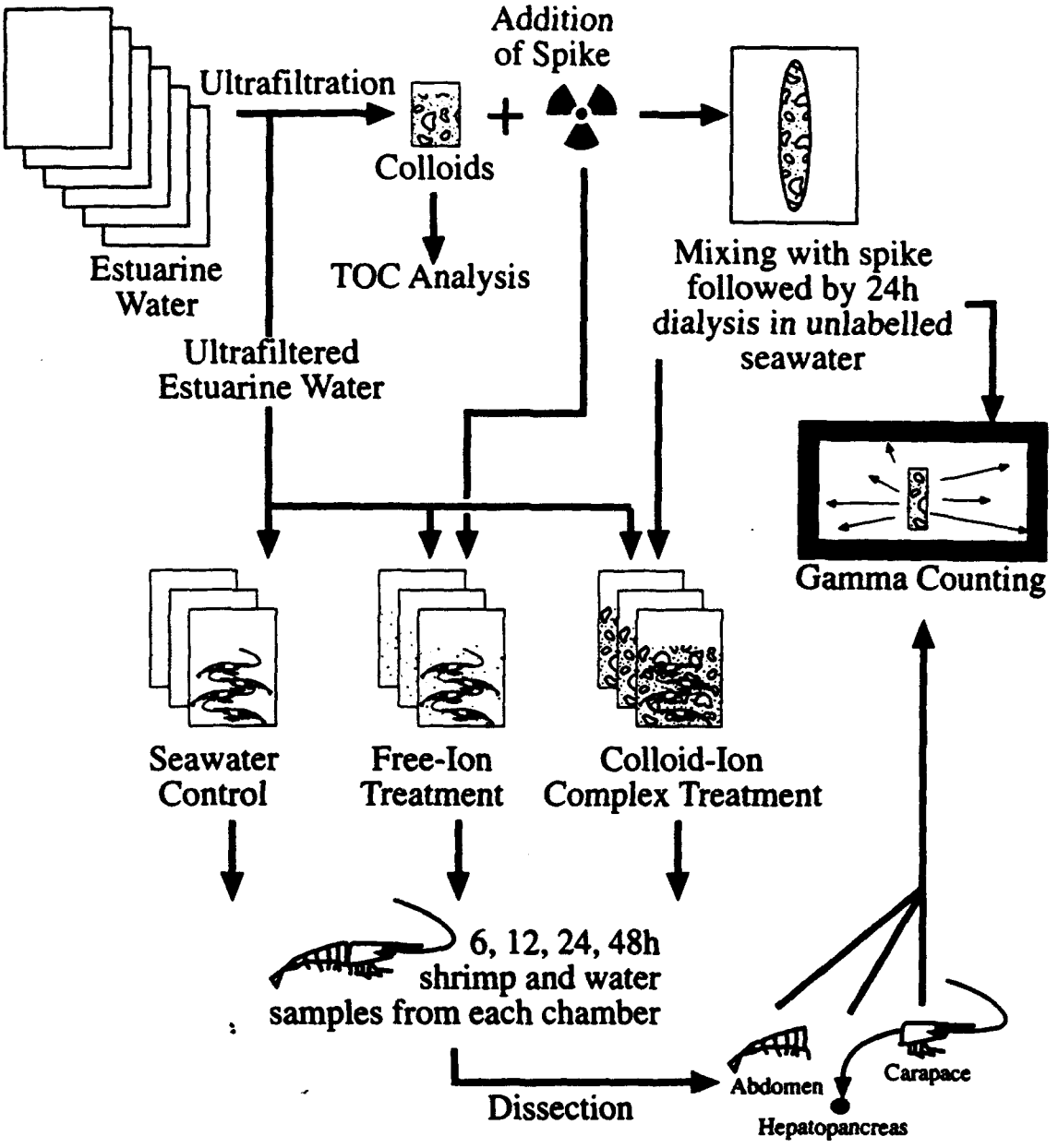

Figure 5. Flow diagram illustrating the Procedures used for conducting colloidally-bound bioavailibility experiments with juvenile white shrimp (P. setiferus).

counting times and improving count precision. Carrier-free or high activity isotopes are being secured for the remaining uptake studies.

Personnel

Although Dr. Mark Benfield left Texas A&M University for the Woods Hole Oceanographic Institution in September 1993, he continues to be directly involved in the project through a subcontract. He visited Galveston for 10 days in January and 32 days in June-July to supervise the experiments conducted by our graduate student (Ms. Roberta Carvalho) and technicians. Mark will continue to visit and remain in close contact with our group until the successful completion of the research.

III. CHARACTERIZATION OF ESTUARINE COLLOIDAL MATTER

Surface Chemistry Of Estuarine Colloidal Matter

The initial phase of this portion of the project consists of potentiometric titration's of colloidal material which were carried out by Dr. Bruce Honeyman (Colorado School of Mines) as part of a collaborative project.

Titration's of marine colloidal organic material (COM) from Galveston Bay were conducted under a nitrogen atmosphere in carbon dioxide depleted demineralized water (Nanopure water) using a custom-designed potentiometric titrator (Redded Rocket, George Redden Enterprises, Menlo Park, CA). Solutions containing the desired amount of COM (150 mg/L) are titrated with CO_2-free standardized NaOH or HCl in a double-walled, closed 50 mL Celstir titration flask. Temperature is kept constant at 25 °C by circulation of water through the outer chamber of the titration flask. Ionic strength is adjusted with $NaCLO_4$. Minimum volumes of titrant (0.001 mL) are dispensed into the COM dispension with computer controlled Gilmont burettes. Suspension pH is monitored with an Orion pH meter/Ross combination electrode and data are stored on disk.

Application of the site affinity distribution function (SADF) approach of Buffle et al. (1990) yielded three component acids with conditional pK_a's of 4.8, 7.1, and 9.6. The pK_a of 7.1 may be anomalous; however, it consistently has appeared in all COM titrations that we have conducted. This acidity distribution is similar to that of COM isolated from shelf waters of the Gulf of Mexico. The total proton concentration of Galveston Bay COM is 0.9 mol/kg.

The Partitioning Of Trace Metals And Metalloids To Estuarine Colloidal Matter

The uptake of trace metals and metalloids to colloidal material from Galveston Bay using radiotracer methodology is scheduled to begin late this summer or early fall.

REFERENCES

Bruland, K.W., K.H. Coale, and L. Mart (1985). Analysis of sea water for dissolved cadmium, copper, and lead: An intercomparison of voltammetric and atomic absorption methods. *Marine Chemistry*, **17**: 285-300.

Buffle, J., R.S. Altman, M. Filella, and A. Tessier (1990). Complexation by natural heterogeneous compounds: site occupation distribution functions, a normalized description of metal complexation. *Geochimica et Cosmochimica Acta*, **54**: 1535-1554.

Flegal, A. R., G.J. Smith, G.A. Gill, S. Sanudo-Wilhelmy and L.C.D. Anderson (1991). Dissolved trace element cycles in the San Francisco Bay Estuary. *Marine Chemistry*, **36**: 329-363.

Landing, W.M. and B. L. Lewis (1992). Collection, processing and analysis of marine particulate and colloidal material for transition metals. *In*: Marine particles: analysis and characterization. D.C. Hurd and D.W. Spencer (editors), American Geophysical Union, pp. 263-272.

Pai S. C., P.Y. Whung, and R.L. Lai (1988). Preconcentration efficiency of Chelex-100 resin for heavy metals in sea water. Part 1. Effects of pH and salts on the distribution ratios of heavy metals. *Anal. Chim. Acta*, 211: 257-270.